

u-Dish 35 mm, low Grid-500

Instructions



The ibidi product family is comprised of a variety of μ -Slides and μ -Dishes, which have all been designed for high-end microscopic analysis of fixed or living cells. The high optical quality of the material is similar to that of glass, so you can perform all kinds of fluorescence experiments with uncompromised resolution and choice of wavelength.

The μ -Dish $^{35\,\text{mm, low}}$ allows you to perform high resolution microscopy in a 35 mm Petri–dish with 7 mm walls. The low height makes high numerical apertures of Köhler illumination possible and provides large access for micromanipulation. The lid can be closed to hinder evaporation during long term experiments.

The Grid-500 is a grid structure for relocating events. It provides 400 distinguishable observation squares of 500 μ m edge length. The grid is clearly visible by phase contrast microscopy and based on the high quality ibidi Polymer Coverslip Bottom. The outer dimensions are identical to ibidi μ -Dishes.

Material

ibidi μ -Slides, μ -Dishes, and μ -Plates are made of a polymer that has the highest optical quality. The polymer coverslip on the bottom exhibits extremely low birefringence and autofluorescence, similar to that of glass. Also, it is not possible to detach the bottom from the upper part. The μ -Slides, μ -Dishes, and μ -Plates are intended for one-time use and are not autoclavable, since they are only temperature-stable up to 80°C/175°F. Please note that gas exchange between the medium and the incubator's atmosphere occurs partially through the polymer coverslip, which should not be covered.

Optical Properties ibidi Polymer Coverslip

Refractive index n _D (589 nm)	1.52
Abbe number	56

Thickness No. 1.5 (180 μm)

Material Polymer coverslip

Please note! The ibidi Polymer Coverslip is compatible with certain types of immersion oil only. A list of suitable oils can be found on page 4.

Geometry

Geometry of the µ-Dish 35 mm, low		
Diameter dish	35 mm	
Volume	800 µl	
Growth area	3.5 cm^2	
Diameter growth area	21 mm	
Coating area using 400 µl	4.1 cm^2	
Height with / without lid	9 mm / 7 mm	
Bottom	ibidi Polymer Coverslip	

Shipping and Storage

The μ -Slides, μ -Dishes and μ -Plates are sterilized and welded in a gas-permeable packaging. The shelf life under proper storage conditions (in a dry place, no direct sunlight) is listed in the following table.

Conditions		
Ambient		
RT (15–25°C)		
Shelf Life		
36 months		

Geometry of the Grid-500

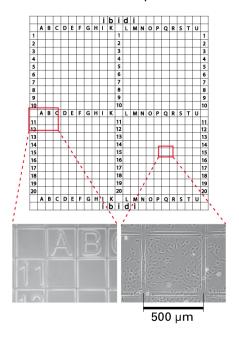
Geometry of the Grid-500		
Number of squares	400	
Repeat distance	$500 \mu m (\pm 1 \%)$	
Groove width	$40\mu m (\pm 5\mu m)$	
Groove depth	$< 5\mu m$	

The four major squares are separated in 10×10 observation fields and indicated by letters and numbers ranging from:

- A to K (I not used) and 1 to 10
- A to K (J not used) and 11 to 20
- L to U and 1 to 10
- L to U and 11 to 20



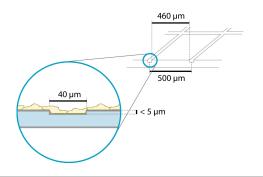
4 x 10 x 10 squares



Characteristics of the Grid

The Grid-500 is made of small grooves inside the ibidi Polymer Coverslip surface of ibidi μ -Dishes. The structure is imprinted on the side on which cells are growing and does not effect cell growth, coating protocols, or surface properties. Proliferation and cell behavior is comparable with standard non–gridded dishes. Cells and grid are in one focal plane.

The grid is made of grooves, which are $40 \, \mu m \ (\pm 5 \, \mu m)$ wide and $< 5 \, \mu m$ deep. Cells can grow in the grooves as well. We recommend using objective lenses up to 20×10^{10} . Anyhow, the optical quality meets the requirements of $63 \times 100 \times 10^{10}$ and 100×10^{10} objective lenses as well (ibidi Polymer Coverslip, No. 1.5).



Surface

The tissue culture-treated ibiTreat surface is a physical surface modification and optimized for adhesion of most cell types. ibiTreat is our most recommended surface modification, because most adherent cells grow well on this hydrophilic version of the ibidi Polymer Coverslip, without the need for any additional coating.

Coating

Detailed information about coatings is provided in Application Note 08: Coating protocols for ibidi labware products

In short, specific coatings are possible following this protocol:

- 1. Prepare your coating solution according to the manufacturer's specifications or reference.
- 2. Apply 400 µl and leave at room temperature for at least 30 minutes.
- 3. Aspirate the solution and wash with the recommended protein dilution buffer.
- 4. The μ-Dish ^{35 mm,low} Grid-500 is ready to be used. Optionally let dry at room temperature. Attention, some coating proteins might degenerate when drying!

Seeding Cells

Depending on your cell type, application of a $4-9 \times 10^4$ cells/ml suspension should result in a confluent layer within 2–3 days.

- Trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration.
- Apply 400 μl cell suspension into the inner well of the μ-Dish. Avoid shaking as this will result in inhomogeneous distribution of the cells.
- After cell attachment add additionally 400 µl of pure medium to ensure optimal grow conditions.
- Cover the μ -Dish with the supplied lid. Incubate at 37°C and 5 % CO₂ as usual.

We do not recommend filling more than 800 μl into the μ-Dish ^{35 mm, low} Grid-500 in order to avoid the liquid contacting the lid.

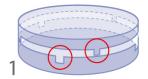
Undemanding cells can be left in their seeding medium for several days and grow to confluence there. However, best results are achieved when the medium is changed every

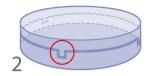


2–3 days. Carefully aspirate the old medium and replace it by up to $800 \mu l$ fresh medium.

Using The Lid

Instructions





- 1. Open position, easy opening
- 2. Close position, for long term studies, minimal evaporation

Tip:

You can stack the μ -Dishes to save space in your incubator. This will not affect cell growth. We recommend making batches with up to 6 μ -Dishes, due to stability reasons. Placing the μ -Dishes into larger Petri dishes simplifies transport and prevents evaporation, heat loss, and contamination when the incubator is opened.

μ-Dish 35 mm Selection Guide

μ-Dish ^{35 mm, low}	μ-Dish ^{35 mm, high}
Low walls (7 mm) for large access to the cells. Designed for micromanipulation and microinjection.	-
0.8 ml 7 mm	2 ml 12 mm

Microscopy

To analyze your cells, no special preparations are necessary. Cells can be directly observed live or fixed, preferably on an inverted microscope. The bottom cannot be removed. For optimal results in fluorescence microscopy and storage of fixed and stained samples, ibidi provides a mounting medium (50001) optimized for μ -Dishes, μ -Slides, and μ -Plates.

Chemical Compatibility

The following table provides some basic information on the chemical and solvent compatibility of the μ -Dish $^{35\,\text{mm},\,\text{low}}$ Grid-500. For a full list of compatible solvents and more information on chemical compatibility, please visit the FAQ section on ibidi.com.

Chemical / Solvent	Compatibility
Methanol	yes
Ethanol	yes
Formaldehyde	yes
Acetone	no
Mineral oil	no
Silicone oil	yes
Immersion oil	See Immersion Oil on page 4.

Minimizing Evaporation

Using the $\mu\text{-Dish}$ with a closed lid, the evaporation in an incubator system with 37°C and 95% humidity is around 1% per day. Using the $\mu\text{-Dish}$ with a closed lid in a 37°C heating system with low humidity (between 20% and 40%), the evaporation is around 10% per day. For reducing the evaporation down to 1% per day in all systems, we recommend sealing the lid with ibidi Anti-Evaporation Oil (50051).



$\mu\text{-Dish}~^{35\,\text{mm},\,\text{low}}$ Grid-500

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Immersion Oil

When using oil immersion objectives with the ibidi Polymer Coverslip, use only the immersion oils specified in the table below. The use of any non-recommended oil could damage the ibidi Polymer Coverslip. The resulting leakage may harm objectives and microscope components. All immersion oils that are not listed in the table below should be considered as non-compatible.

Company	Product	Ordering No.	Lot Number	Test Date
ibidi	ibidi Immersion Oil	50101	16-12-27	01/2017
Cargille	Type A	16482	100592	01/2017
Cargille	Type HF	16245	92192	01/2017
Carl Roth	Immersion oil	X899.1	414220338	01/2017
Leica	Immersion Liquid	11513859	n.a.	03/2011
Nikon	Immersion Oil F2 30cc	MXA22192	n.a.	01/2020
Nikon	Silicone Immersion Oil 30cc	MXA22179	20191101	01/2020
Olympus	Silicone Immersion Oil	SIL300CS-30CC	N4190800	01/2017
Zeiss	Immersol 518 F	444960	160706	01/2017
Zeiss	Immersol W 2010	444969	101122	04/2012



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μ -Dish $^{35\,mm,\,low}$ Grid-500

Ordering Information

μ -Dish $^{35\,mm,\,low}$



Cat. No.	Description
80136	μ -Dish $^{35 \text{ mm, low}}$ ibiTreat: \emptyset 35 mm, low wall (800 μ l volume), #1.5 polymer coverslip, tissue culture treated, sterilized
80131	μ -Dish ^{35 mm, low} Uncoated: Ø 35 mm, low wall (800 μl volume), #1.5 polymer coverslip, hydrophobic, sterilized

μ -Dish $^{35 \text{ mm, low}}$ Grid-500



Cat. No.	Description
80156	μ-Dish ^{35 mm, low} Grid-500 ibiTreat : Ø 35 mm, low wall (800 μl volume), grid repeat distance
	500 μm, #1.5 polymer coverslip, tissue culture treated, sterilized

Culture-Insert in μ -Dish $^{35 \text{ mm, low}}$



Cat. No.	Description
80206	Culture-Insert 2 Well in μ -Dish 35mm,low , ibiTreat: ready-to-use, tissue culture treated, sterilized

For research use only!

Further information can be found at www.ibidi.com. For questions and suggestions please contact us by e-mail *info@ibidi.de* or by telephone +49 (0)89/520 4617 0.

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